Request for Biomarkers

Background

Biomarkers are defined as "a characteristic that is measured and evaluated as an indicator of normal biologic processes, pathogenic processes, or pharmacologic responses to therapeutic interventions". A major priority of the Early Detection Research Network (EDRN) is the selection and rapid validation of biomarkers. To address this priority, organ-focused groups have been organized with the goal of reviewing the status of potential biomarkers, individually and as potential panels of biomarkers.

Opportunities for Support of Translational Validation of Biomarkers

The GI Collaborative of the EDRN solicits potential biomarkers for the early detection of colorectal neoplasia from the research community. A product may be proposed by any individual investigator, group or corporate entity. The EDRN offers a high quality human biosample resource consisting of samples collected from 230 patients with colorectal adenocarcinoma, 334 patients with colonic polyps, 34 patients with inflammatory bowel disease, and 350 patients with endoscopically proven normal colons. Samples from approximately 25 to 30 new patients are collected monthly. The current sample bank consists of aliquots of human serum and plasma (200 microliters), urine (5 ml aliquots), DNA from white blood cells, and matched paraffin tissue sections. A total of over 25,000 aliquoted samples are available. The samples are collected using standard operating procedures that include bar coded tracking, a web-front ended relational database, and data elements that have been piloted and validated for EDRN use. The samples are managed in a professional biosample repository at -80°C in freezers that are sensor controlled, backed up with diesel generators, and automated call in system.

The GI Collaborative will provide multidisciplinary expertise to investigators or research groups with novel products with high potential from any source that may enhance the early diagnosis or risk identification of colorectal neoplasia (adenocarcinoma, adenoma, dysplastic aberrant crypt foci). The Collaborative will provide high quality epidemiology and biostatisics expertise for the design, implementation and analysis of prevalidation and validation studies aimed at demonstrating diagnostic efficacy of such novel products. The EDRN will provide experienced analytical support including scale up methodologies using state of the art technology. The analytical support is performed to CLIA quality control standards, thus ensuring future regulatory approval should a product prove effective.

The EDRN offers financial support for support for translational validation with the aim of demonstrating efficacy for early diagnosis or risk assessment for colorectal neoplasia of those biomarkers selected by the GI Collaborative review group that are considered highly innovative and potentially effective diagnostics for colorectal neoplasia.

Process

1. Complete the attached submittal form that consists of the following elements:

a. Investigator/Research Group name and contact information

b. Biomarker metrics

c. Two page proposal that briefly and succinctly follows the organizational structure:

i. Background: Describe the theoretical and then practical basis of the proposed biomarker target. Provide preclinical support for the proposed target or rationale for a profile that does not have a direct mechanistic rationale. There is no need to justify research into the early detection of colorectal neoplasia.

ii. Technological Approach: Describe the technology as it is applied to the biomarker proposed for development. Provide preliminary evidence of reproducibility and potential scale-up of an assay.

iii. Preliminary Data: Describe preliminary studies in rodent models and with human biosamples. These data need not be extensive or conclusive but must provide evidence of innovation and potential usefulness in humans. The biomarker must have sufficient preliminary data to qualify for a prevalidation trial (see Attachment #2: "Colon Validation Standards").

2. Submit the proposal to Dr. Paul Wagner (wagnerp@mail.nih.gov). ELECTRONIC SUBMISSIONS ONLY.

3. DEADLINE FOR SUBMISSIONS: October 1, 2005

Review Process

1. All submissions will be reviewed by the Colon Cancer Biomarkers Group of the EDRN's GI Collaborative. Submissions will be reviewed by at least two members of the group and assigned a NIH-type merit rating. Each submission will then be discussed by the reviewers and a NIH review score will be assigned to each proposal.

2. Expected Completion of Review:
November 15, 2005.

Outcome of Review Process

Investigators submitting successful applications will be invited to a meeting sponsored by the EDRN to review their biomarkers in more detail. At that meeting, biomarkers will be reviewed by members, Associate Members, and invited investigators as a panel to select biomarkers for EDRN Support for prevalidation and validation projects.

ATTACHMENT #1: Colorectal Biomarker Submittal Form

EARLY DETECTION RESEARCH NETWORK
BIOMARKER SUBMITTAL FORM

IDENTIFICATION

Name of Biomarker

Investigator:
Organization:

Address:

Telephone Number:
Email Address:

BIOMARKER METRICS
Instructions: Please provide brief summary data regarding the current status of your biomarker(s).

1. Analytical Method Used to Detection Biomarker:

2. Preliminary Data with Animal Models:
a. Animal Model Used:
b. Carcinogen or implanted tumor used:
c. Biomarker in Controls (mean±SD):

d. Biomarker in Tumor/Carcinogen (mean±SD):

3. Preliminary Data in Humans
a. Type of Biosamples:
b. Tumor/Precancer:
c. Number of Controls:
d. Number of Cancer/Precancers:
e. Biomarker in Controls (mean±SD):

f. Biomarker in Cancer/Precancer (mean±SD):

g. Comments regarding performance of biomarker, potential use:

ATTACHMENT #2

A RATIONALE

A.1 Concepts of Biomarker Validation

There are 3 fundamental concerns related to clinical biomarker validation (1, 2):
(1) ***Overfitting***. Are results (discrimination) due to chance (e.g., overfitting of a multivariable model, without checking for reproducibility)? This refers to the tendency of models trained on large numbers of variables measured on small numbers of observations to produce extraordinarily high sensitivity and specificity, and then fail on independent validation sets (2-5).
(2) ***Bias***. Are results due to differences between the cancer and the control *samples* that do not exist in the cancer and control *populations*? This refers to misidentification of the cause of differences between samples, for instance, if a sample of patients is much older than a sample of controls, then differences due to age may be misattributed to disease (6, 7).
(3) ***Generalizability***. Are results generalizable to appropriate clinical populations? This refers to the similarity of the distribution of markers or sets of markers between the samples studied, and samples derived from a larger clinical or screening population.

A.2 Current Barriers in the EDRN Towards Addressing these Concepts

The EDRN has formalized biomarker validation procedures using a phased approach (8). Nevertheless, despite the discovery of a large number of biomarkers for the detection and risk assessment of common epithelial cancers, few have progressed to large cross-sectional or longitudinal validation trials in humans. Moving a biomarker beyond the first, discovery, phase to validation phases remains difficult because moving to Phase II or III depends upon statistical evidence of predictive power and robustness in a sample set that is representative of the clinical population.

A.3 Reference Sets to Overcome the Barriers

The GI Collaborative proposes establishment of reference sample sets for prevalidation and validation to speed validation of promising biomarkers for cancer early diagnosis and risk assessment.

A.4 Definition

A reference set comprises biosamples collected under good clinical practice (GCP) conditions that are sufficient to permit decision analysis with statistical precision.

A.5 Good Clinical Practice Conditions

Good Clinical Practice is defined as collection of human biosamples and accompanying data elements using Standard Operating Procedures. Such procedures are defined in advance in a written protocol document and enforced through internal and external auditing mechanisms.

A.6 Management of Reference Sets

Preliminary data, defined in advance, are required to trigger use of a reference set. The quantity and quality of the preliminary data vary with the proposed biomarker indication and estimate of clinical impact. Agreements regarding publications, data management, sample release, and analytical quality control are made in advance. Samples are assayed based upon scientifically rigorous design. Data from both open and closed labeled assays are analyzed by biostatisticians who have no vested interest in success or failure of a given product.

B SUMMARY OF STATE OF THE ART: EARLY DETECTION OF COLORECTAL NEOPLASIA

B.1 Current State of the Art, Serum Based Biomarkers for Colorectal Neoplasia

Most serum-based biologic biomarkers are derived from known overexpression or mutated signaling pathways associated with colorectal adenocarcinoma. The biomarkers can be grouped into several broad categories including genetic markers, growth factors, immunologic products, and angiogenesis factors (9-12). To date, the direct detection of aberrant genes or genetic material specific to colorectal neoplasms (e.g. APC, β-catenin, *K-ras*, DCC, and p53) has been limited by the technical challenge of DNA recovery, the large number of potential underlying genetic mutations, and by the limited sensitivity of any single genetic alteration. (13-17). However, recent reports suggest that detection of *K-ras* mutations alone (18, 19) or in combination with p16 methylation (20) have shown promise for differentiating and risk-stratifying patients with colorectal adenocarcinoma. The ability to detect epigenetic events with methylation-specific PCR may greatly improve the performance of any panel of genetic biomarkers (16, 21-23).

High serum concentrations of insulin-like growth factors (IGF) and low levels of their binding proteins have been shown to correlate with colorectal adenocarcinoma risk in large cohort studies (24-26). However, this correlation has recently been disputed (27), and the associated relative risks are low. Loss of IGF2 imprinting appears to be significantly more common in individuals with colorectal neoplasms or even a family history of colorectal adenocarcinoma (28-31). Other growth factors recently studied include TGF-β1 (28-34) and VEGF (35, 36). The association of these markers with risk of colorectal adenocarcinoma does not appear to be strong enough to warrant our attention at this point.

Since the discovery of CEA (37, 38), investigators have searched for immunological biomarkers that are both sensitive and specific for colorectal adenocarcinoma. Recent advances include detection of CEA mRNA (39, 40) and CEA splice variants as well as novel and combination panels of cancer and carbohydrate antigens and antibodies (41-50) and even soluble interleukin-2 receptors (51).

Angiogenesis factors that may serve as biomarkers for colorectal adenocarcinoma include angiogenin (52), endostatin (53), and endothelins (54, 55). Of the matrix metalloproteinases (56-58), plasma TIMP1 levels have been shown to be elevated in colorectal adenocarcinoma but low in normal subjects and patients with other inflammatory or malignant conditions (sensitivity of 63% and a specificity of 98% for diagnosis of colorectal adenocarcinoma) (59). Other potential biomarkers include cell adhesion molecules (60), and nuclear mitotic apparatus proteins (61, 62).

B.2 Rationale and Current State of the Art, Stool Based Biomarkers for Detection of Colorectal Neoplasia

Wide scale screening using fecal occult blood tests (FOBT) results in 15-33% reduction in colorectal adenocarcinoma mortality, but at the expense of many unneeded colonoscopies (63-66). Despite specificity of approximately 95%, reasonable cost effectiveness of $7,200 incremental costs per life-year gained (67), FOBT's sensitivity ranging from 15 to 30% (64, 66, 68) leave room for substantial improvement. The addition of sigmoidoscopy to FOBT increases the detection of adenomas by approximately two fold over FOBT alone but there are no data demonstrating enhanced mortality reduction (68). Colonoscopy, with estimated sensitivity

exceeding 90% or adenocarcinoma and large adenoma and specificity exceeding 99% requires a thorough bowel preparation and sedation, causes patient discomfort, small but non-negligible risk of complications (68-74) has a reported cost-benefit of $17,010 incremental costs per life-year gained (67). The cost, morbidity, and burdens upon the medical care system if large scale colonoscopic screening were to be generalized to large populations provide a strong rationale for early detection biomarkers that can be easily and cost-effectively deployed.

Since the neoplastic transformation process of the colonic epithelium results in cells shedding into the stool, collection of fecal material is likely to yield detectable molecular and biochemical events associated with cellular transformation (75). Fecal sample testing using molecular diagnostic tests are emerging as potentially important new approaches that have the potential of providing the cost-effective, sensitive early detection of colorectal neoplasia. Details of many of the currently employed and novel approaches have been recently reviewed (76). Because a single genetic product is unlikely to have sufficient detection sensitivity and specificity to be used as a "stand-alone" diagnostic test (76), Exact Sciences Corporation has designed a fecal based DNA detection system, PreGen-Plus, that exploits the concept of chromosomal instability with mutations progressively accumulating in the adenomatous polyposis coli, p53 tumor suppressor genes and the K-*ras* oncogene. PreGen-Plus detects 28 specific mutations in the APC, p53, and *K-ras* genes, deletions in BAT26, and other genomic changes associated with sporadic colorectal combined in a DNA-based stool assay (75-78) (79). This test relies upon preservation of naked DNA in human stool samples. The test requires a large volume fecal sample from which purified DNA is prepared using oligonucleotide based hybrid capture. The genetic mutation sites are detected and quantified with real time PCR (79). Preliminary publications in small trials (16 to 65 subjects) reported test sensitivity ranging from 62 to 91% for adenocarcinoma detection and 27 to 82% for adenoma detection with specificity ranging from 93 to 98% (76). Validation of these preliminary data in a large (4,404 evaluated subjects), prospective colorectal cancer screening trial resulted in a sensitivity of 51.6% (95% CI=34.8-68.0%) for detection of adenocarcioma; 15.1% (95% CI=12.0-10%) for detection of adenomas ≥1 cm with double the sensitivity when the adenoma had dysplasia. Specificity for the fecal DNA test was 95.2% (79). These data support the concept that fecal DNA tests can enhance sensitivity to FOBT and serve as an intermediate, noninvasive screening tool for colorectal adenocarcinoma. The cost-benefit Markov model employed by Song et al, assumed the diagnostic sensitivity of the multi-gene fecal DNA panel to be 65% for adenocarcinoma and 40% for colorectal adenomas and a base cost estimate of $695 per PreGen-Plus test. The model generated a cost of $47,700 incremental life-year gained compared to the natural history. In order for a fecal DNA to become cost effective, the base cost would need to be reduced to $195 per test using the sensitivity assumptions that were 10% higher than the recently published prospective screening trial (67) or the sensitivity substantially enhanced.

C STANDARDS FOR RELEASE OF SAMPLES FROM THE REFERENCE SET

Sample allocation to EDRN investigators must be fair and data-based. The GI Collaborative proposes procedures that are equitable yet preserve this valuable resource. The following are standards applied for release of samples for prevalidation and for validation of biomarkers intended for early diagnosis and risk assessment of colorectal adenocarcinoma.

C.1 Prevalidation: Biomarkers intended as screening biomarkers for colorectal adenocarcinoma

A putative biomarker need address any of the detection endpoints (carcinoma or adenoma) to be eligible for access to prevalidation sample sets.

B.1.1 Biomarkers intended to screen normal individuals for colonoscopy

These biomarkers are generally serum, urine, or stool based tests that are noninvasive and intended to select those individuals who require more invasive or expensive screening or resection of adenomas via colonoscopy.

B.1.2 Preliminary data required to access prevalidation sample sets

A putative biomarker need address any of the detection endpoints (carcinoma or adenoma) to be eligible for access to prevalidation samples sets.

Detection of colorectal adenocarcinoma: To access serum, plasma, DNA, urine or paraffin section reference set biosamples for which multiple aliquots will be available, a biomarker must demonstrate, in a small, preliminary prediction analysis, a sensitivity of 40% and specificity of 80% prediction of any stage adenocarcinoma of the colon.

Detection of adenoma, any size: To access serum, plasma, DNA, urine or paraffin section reference set biosamples for which multiple aliquots will be available, a biomarker must demonstrate, in a small, preliminary prediction analysis, a sensitivity of 30% and specificity of 70% prediction of adenoma.

Quality of preliminary data: The sample set used to generate the preliminary data may be small (10 subjects per diagnostic group), biased, and collected from local, non-GCP sources.

Access to frozen tissue samples: Frozen biosamples are small (4 mm) and limited (5 to 10 samples per subject). It is unclear whether frozen tissue samples will be necessary at the prevalidation level. Access to frozen biopsies from the reference set are more stringent—sensitivity=70%, specificity=90% from preliminary data sets.

C.2 Validation: Biomarkers intended as biomarkers for colorectal adenocarcinoma

A putative biomarker need address any of the detection endpoints (carcinoma or adenoma) to be eligible for development and approval of validation trial. Biomarkers that are capable of detecting adenomas will be given priority over biomarkers that detect only carcinomas. Urine based biomarkers will be prioritized over serum based biomarkers which will be prioritized over stool based biomarkers.

B.1.1 Biomarkers intended to screen normal individuals for colonoscopy

These biomarkers are generally serum, urine, or stool based tests that are noninvasive and intended to select those individuals who require more invasive or expensive screening or resection of adenomas via colonoscopy.

B.1.2 Preliminary data from prevalidation sample set to justify an EDRN Core Supported Validation Project

Detection of colorectal adenocarcinoma, early stage (I or II): To be competitive for EDRN support in a Core supported validation trial, a biomarker must demonstrate a minimum sensitivity of 50% and a minimum specificity of 90% prediction of early stage (stage I or II) adenocarcinoma of the colon, after a blinded test set assay that is analyzed by an EDRN biostatistical consultant.

Detection of adenoma, any size: To be competitive for EDRN support in a Core supported validation trial, a biomarker must demonstrate a minimum sensitivity of 50% and a minimum specificity of 90% prediction of the presence of a colorectal adenoma of any size, after a blinded test set assay that is analyzed by an EDRN biostatistical consultant.

Analytical Platform: To be competitive for EDRN support in a Core supported validation trial, the technology necessary for assay of the biomarker must be adaptable to scale up and reproducibility study by an EDRN Biomarker Reference Laboratory (BRL).

References

1. Feinstein A. Multivariable Analysis: An Introduction. New Haven, CT: Yale University Press; 1996.
2. Ransohoff DF. Rules of evidence for cancer molecular-marker discovery and validation. Nat Rev Cancer 2004;4(4):309-14.
3. Stone M. Cross-validatory choice and assessment of statistical predictions. J Royal Stat Soc 1974;36:111-147.
4. Ransohoff DF. Discovery-based research and fishing. Gastroenterology 2003;125(2):290.
5. Ransohoff DF. Gene-expression signatures in breast cancer. N Engl J Med 2003;348(17):1715-7; author reply 1715-7.
6. Baggerly KA, Morris JS, Coombes KR. Reproducibility of SELDI-TOF protein patterns in serum: comparing datasets from different experiments. Bioinformatics 2004;20(5):777-85.
7. Sorace JM, Zhan M. A data review and re-assessment of ovarian cancer serum proteomic profiling. BMC Bioinformatics 2003;4(1):24.
8. Sullivan Pepe M, Etzioni R, Feng Z, Potter JD, Thompson ML, Thornquist M, et al. Phases of biomarker development for early detection of cancer. J Natl Cancer Inst 2001;93(14):1054-61.
9. Srivastava S, Verma M, Henson DE. Biomarkers for early detection of colon cancer. J Natl Cancer Inst 2001;7:1118-1126.
10. McDermott U, Longley DB, Johnston PG. Molecular and biochemical markers in colorectal cancer. Ann Oncol 2002;13 Suppl 4:235-45.
11. Negm RS, Verma M, Srivastava S. The promise of biomarkers in cancer screening and detection. Trends Mol Med 2002;8(6):288-93.
12. Hill MJ. Molecular and clinical risk markers in colon cancer trials. Eur J Cancer 2000;36(10):1288-91.
13. Hibi K, Robinson CR, Booker S, Wu L, Hamilton SR, Sidransky D, et al. Molecular detection of genetic alterations in the serum of colorectal cancer patients. Cancer Res 1998;58(7):1405-7.
14. Vlems FA, Diepstra JH, Cornelissen IM, Ligtenberg MJ, Wobbes T, Punt CJ, et al. Investigations for a multi-marker RT-PCR to improve sensitivity of disseminated tumor cell detection. Anticancer Res 2003;23(1A):179-86.
15. Yamaguchi K, Takagi Y, Aoki S, Futamura M, Saji S. Significant detection of circulating cancer cells in the blood by reverse transcriptase-polymerase chain reaction during colorectal cancer resection. Ann Surg 2000;232(1):58-65.
16. Zou H, Yu B, Wang Z, Sun J, Cang H, Gao F, et al. Detection of aberrant p16 methylation in the serum of colorectal cancer patients. Clin Cancer Res 2002;8:188-191.
17. Noh YH, Im G, Ku JH, Lee YS, Ahn MJ. Detection of tumor cell contamination in peripheral blood by RT-PCR in gastrointestinal cancer patients. J Korean Med Sci 1999;14(6):623-8.
18. Borchers R, Heinzlmann M, Zahn R, Witter K, Martin K, Loeschke K, et al. K-ras mutations in sera of patients with colorectal neoplasia and long-standing inflammatory bowel disease. Scand J Gastroenterol 2002;37(6):715-718.

19. Salbe C, Trevisiol C, Ferruzzi E, Mancuso T, Nascimbeni R, Di Fabio F, et al. Molecular detection of codon 12 K-RAS mutations in circulating DNA from serum of colorectal cancer patients. Int J Biol Markers 2000;15(4):300-307.
20. Lecomte T, Berger A, Zinzindohoue F, Micard S, Landi B, Blons H, et al. Detection of free-circulating tumor-associated DNA in plasma of colorectal cancer patients and its association with prognosis. Int J Cancer 2002;100(5):542-8.
21. Grady WM, Rajput A, Lutterbaugh JD, Markowitz SD. Detection of aberrantly methylated hMLH1 promoter DNA in the serum of patients with microsatellite unstable colon cancer. Cancer Res 2001;61(3):900-2.
22. Nakayama H, Hibi K, Taguchi M, Takase T, Yamazaki T, Kasai Y, et al. Molecular detection of p16 promoter methylation in the serum of colorectal cancer patients. Cancer Lett 2002;188(1-2):115-119.
23. Verma M, Srivastava S. Epigenetics in cancer: implications for early detection and prevention. Lancet Oncol 2002;3(12):755-63.
24. Giovannucci E. Insulin-like growth factor-1 and binding protein-3 and risk of cancer. Horm Res 1999;51(Suppl 3):34-41.
25. Giovannucci E, Pollak MN, Platz EA, Willet WC, Stampfer MJ, Majeed N, et al. A prospective study of plasma Insulin-like growth factor and binding protein-3 and risk of colorectal neoplasia in women. Cancer Epidemiol Biomarkers Prev 2000;9(4):345-349.
26. Giovannucci E, Pollak M, Platz EA, Willet WC, Stampfer MJ, Majeed N, et al. Insulin-like growth factor I (IGF-I), IGF-binding protein-3 and the risk of colorectal adenoma and cancer in the Nurses' Health Study. Growth Horm IGF Res 2000;10 Suppl A:S30-31.
27. Palmquist R, Stattin P, Rinaldi S, Biessy C, Stenling R, Riboli E, et al. Plasma insulin, IGF-binding proteins-1 and -2 and risk of colorectal cancer: a prospective study in northern Sweden. Int J Cancer 2003;107(1):89-93.
28. Cruz-Correa M, Cui H, Giardiello FM, Powe NR, Hylind L, Robinson A, et al. Loss of imprinting of insulin growth factor II gene: a potential heritable biomarker for colon neoplasia predisposition. Gastroenterology 2004;126(4):964-70.
29. Cui H, Onyango P, Brandenburg S, Wu Y, Hsieh CL, Feinberg AP. Loss of imprinting in colorectal cancer linked to hypomethylation of H19 and IGF2. Cancer Res 2002;62(22):6442-6.
30. Cui H, Cruz-Correa M, Giardiello FM, Hutcheon DF, Kafonek DR, Brandenburg S, et al. Loss of IGF2 imprinting: a potential marker of colorectal cancer risk. Science 2003;299(5613):1753-1755.
31. Woodson K, Flood A, Green L, Tangrea JA, Hanson J, Cash B, et al. Loss of insulin-like growth factor-II imprinting and the presence of screen-detected colorectal adenomas in women. J Natl Cancer Inst 2004;96(5):407-10.
32. Tsushima H, Kawata S, Tamura S, Ito N, Shirai Y, Kiso S, et al. High levels of transforming growth factor beta 1 in patients with colorectal cancer: association with disease progression. Gastroenterology 1996;110(2):375-382.
33. Tsushima H, Ito N, Tamura S, Matsuda Y, Inada M, Yabuuchi I, et al. Circulating Transforming Growth Factor beta-1 as a predictor of liver metastasis after resection in colorectal cancer. Clin Cancer Res 2001;7:1258-1262.
34. Narai S, Watanabe M, Hasegawa H, Nishibori H, Endo T, Kubota T, et al. Significance of Transforming growth factor beta 1 as a new tumor marker for colorectal cancer. Int J Cancer 2002;97(4):508-511.

35. Broll R, Erdmann H, Duchrow M, Oevermann E, Schwandner O, Markert U, et al. Vascular endothelial growth factor (VEGF) -- a valuable serum tumour marker in patients with colorectal cancer? Eur J Surg Oncol 2001;27(1):37-42.
36. Takeda A, Shimada H, Imaseki H, Okazumi S, Natsume T, Suzuki T, et al. Clinical significance of serum vascular endothelial growth factor in colorectal cancer patients : correlation with clinicopathological factors and tumor markers. Oncol Rep 2000;7(2):333-338.
37. Gold P, Freedman SO. Specific carcinoembryonic antigens of the human digestive system. J Exp Med 1965;122:467-481.
38. Gold P, Freedman SO. Demonstration of tumor specific antigens in human colonic carcinoma by immunologic tolerance and absorption techniques. J Exp Med 1965;121:439.
39. Guadagni F, Roselli M, Cosimelli M, Mannella E, Tedesco M, Cavaliere F, et al. TAG-72 (CA 72-4 assay) as a complimentary serum tumor antigen to carcinoembryonic antigen in monitoring patients with colorectal cancer. Cancer 1993;72(7):2098-2106.
40. Taniguchi T, Makino M, Suzuki K, Kaibara N. Prognostic significance of reverse transcriptase-polymerase chain reaction measurement of carcinoembryonic antigen mRNA levels in tumor drainage blood and peripheral blood of patients with colorectal carcinoma. Cancer 2000;89(5):970-976.
41. Carpelan-Holmstrom M, Louhimo J, Stenman UH, Alfthan H, Haglund C. CEA, CA 19-9 and CA 72-4 improve the diagnostic accuracy in gastrointestinal cancers. Anticancer Res 2002;22(4):2311-2316.
42. Nakagoe T, Sawai T, Tsuji T, Jibiki M, Nanashima A, Yamaguchi H, et al. Prognostic value of circulating sialyl Tn antigen in colorectal cancer patients. Anticancer Res 2000;20(5C):3863-3869.
43. Nakagoe T, Sawai T, Tsuji T, Jibiki MA, Nanashima A, Yamaguchi H, et al. Preoperative serum levels of sialyl Lewis(a), sialyl Lewis(x), and sialyl Tn antigens as prognostic markers after curative resection for colorectal cancer. Cancer Detect Prev 2001;25(3):299-308.
44. Nakagoe T, Sawai T, Tsuji T, Jibiki M, Nanashima A, Yamaguchi H, et al. Circulating sialyl Lewis(x), sialyl Lewis(a), and sialyl Tn antigens in colorectal cancer patients: multivariate analysis of predictive factors for serum antigen levels. J Gastroenterol 2001;36(3):166-172.
45. Sato T, Nishimura G, Nonomura A, Miwa K, Miyazaki I. Serological studies on CEA, CA 19-9, STn and SLX in colorectal cancer. Hepatogastroenterology 1999;46(26):914-919.
46. Pinczower GD, Gianello RD, Williams RP, Preston BN, Linnane AW. Monoclonal antibody 4D3 detects small intestinal mucin antigen (SIMA)-- glycoprotein in the serum of patients with colorectal cancer. Int J Cancer 1993;54(3):391-396.
47. Eskelinen M, Pasanen P, Kulju A, Janatuinen E, Miettinen P, Poikolainen E, et al. Clinical evaluation of serum tumour markers CEA, CA 50 and CA 242 in colorectal cancer. Anticancer Res 1994;14(3B):1427-1432.
48. Spila A, Ferroni P, Cosimelli M, D'Alessandro R, Abbolio MR, Mariotti S, et al. Comparitive analysis of CA 242 and CA 19-9 serum tumor markers in colorectal cancer patients. A longitudinal evaluation. Anticancer Res 2001;21(2B):1263-1270.
49. Nakamura H, Hinoda Y, Nakagawa N, Makiguchi Y, Itoh F, Endo T, et al. Detection of circulating anti-MUC1 mucin core protein antibodies in patients with colorectal cancer. J Gastroenterol 1998;33:354-361.

50. Perez CA, Ravindranath MH, Gupta RK, Tollenaar RA, van de Velde CJ, Wood TF, et al. Serum total gangliosides and TA90-IC levels: novel immunologic markers in colorectal cancer. Cancer J 2002;8(1):55-61.
51. Sakata H, Murakami S, Hirayama R. Serum soluble interleukin-2 receptor (IL-2R) and immunohistochemical staining of IL-2R/Tac antigen in colorectal cancer. Int J Clin Oncol 2002;7(5):312-317.
52. Shimoyama S, Yamasaki K, Kawahara M, Kaminishi M. Increased serum angiogenin concentration in colorectal cancer is correlated with cancer progression. Clin Cancer Res 1999;5(5):1125-1130.
53. Feldman AL, Alexander HR, Jr, Bartlett DL, Kranda KC, Miller MS, Costouros NG, et al. A prospective analysis of plasma endostatin levels in colorectal cancer patients with liver metastases. Ann Surg Oncol 2001;8(9):741-745.
54. Simpson RA, Dickinson T, Porter KE, London NJ, Hemingway DM. Raised levels of plasma big endothelin 1 in patients with colorectal cancer. Br J Surg 2000;87(10):1409-1413.
55. Peeters CF, Thomas CM, Sweep FC, Span PN, Wobbes T, Ruers TM. Elevated serum endothelin-1 levels in patients with colorectal cancer; relevance for prognosis. Int J Biol Markers 2000;15(4):288-293.
56. Pellegrini P, Contasta I, Berghella AM, Gargano E, Mammarella C, Adorno D. Simultaneous measurement of soluble carcinoembryonic antigen and the tissue inhibitor of metalloproteinase TIMP 1 serum levels for use as markers of pre-invasive to invasive colorectal cancer. Cancer Immunol Immunother 2000;49(7):388-394.
57. Yukawa N, Yoshikawa T, Akaike M, Sugimasa Y, Takemiya S, Yanoma S, et al. Plasma concentration of tissue inhibitor of matrix metalloproteinase 1 in patients with colorectal carcinoma. Br J Surg 2001;88(12):1596-1601.
58. Barozzi C, Ravaioli M, D'Errico A, Grazi GL, Poggioli G, Cavrini G, et al. Relevance of biologic markers in colorectal carcinoma: a comparative study of a broad panel. Cancer 2002;94(3):647-57.
59. Holten-Anderson MN, Christensen IJ, Nielsen HJ, Stephens RW, Jensen V, Nielsen OH, et al. Total levels of tissue inhibitor of metalloproteinases 1 in plasma yield high diagnostic sensitivity and specificity in patients with colon cancer. Clin Cancer Res 2002;8(1):156-164.
60. Alexiou D, Karayiannakis AJ, Syrigos KN, Zbar A, Kremmyda A, Bramis I, et al. Serum levels of E-selectin, ICAM-1 and VCAM-1 in colorectal cancer patients: correlations with clinicopathological features, patient survival and tumour surgery. Eur J Cancer 2001;37(18):2392-2397.
61. Briggman J, Genduso R, Camara C, Healy B, Shapiro K, Roos R, et al. NuMA: evaluation of a new biomarker for the detection of low stage colorectal cancer. Anticancer Res 1999;19(4A):2411-2414.
62. Hasholzner U, Stieber P, Zimmermann A, Burges A, Hofmann K, Schmitt UM, et al. Nuclear mitotic apparatus protein (NuMA) in benign and malignant diseases. Anticancer Res 1999;19(4A):2415-2420.
63. Mandel JS, Bond JH, Bradley M, Snover DC, Church TR, Williams S, et al. Sensitivity, specificity, and positive predictivity of the Hemoccult test in screening for colorectal cancers. The University of Minnesota's Colon Cancer Control Study [see comments]. Gastroenterology 1989;97(3):597-600.
64. Mandel JS, Bond JH, Church TR, Snover DC, Bradley GM, Schuman LM, et al. Reducing mortality from colorectal cancer by screening for fecal occult blood. Minnesota Colon

Cancer Control Study [published erratum appears in N Engl J Med 1993 Aug 26;329(9):672] [see comments]. N Engl J Med 1993;328(19):1365-71.
65. Kronborg O. Screening for colorectal cancer with Hemoccult-II in the average risk population. Gastroenterologie Clinique et Biologique 1998;22(3 Suppl):S44-8.
66. Kronborg O, Fenger C, Olsen J, Jorgensen O, Sondergaard O. Randomised study of screening for colorectal cancer with faecal-occult-blood test. Lancet 1996;348:1467-1471.
67. Song K, Fendrick AM, Ladabaum U. Fecal DNA testing compared with conventional colorectal cancer screening methods: a decision analysis. Gastroenterology 2004;126(5):1270-9.
68. Pignone M, Rich M, Teutsch SM, Berg AO, Lohr KN. Screening for colorectal cancer in adults at average risk: a summary of the evidence for the U.S. Preventive Services Task Force. Ann Intern Med 2002;137(2):132-41.
69. Winawer SJ. Appropriate intervals for surveillance. Gastrointestinal Endoscopy 1999;49(3 Pt 2):S63-6.
70. Winawer SJ, Fletcher RH, Miller L, Godlee F, Stolar MH, Mulrow CD, et al. Colorectal cancer screening: clinical guidelines and rationale [see comments] [published errata appear in Gastroenterology 1997 Mar;112(3):1060 and 1998 Mar;114(3):625]. Gastroenterology 1997;112(2):594-642.
71. Winawer SJ, St John DJ, Bond JH, Rozen P, Burt RW, Waye JD, et al. Guidelines for the prevention of colorectal cancer: update based on new data. World Health Organization Collaborating Center for the Prevention of Colorectal Cancer]. Zeitschrift fur Gastroenterologie 1995;33(9):574-6.
72. Winawer SJ, Zauber AG, Ho MN, O'Brien MJ, Gottlieb LS, Sternberg SS, et al. The National Polyp Study. Eur J Cancer Prev 1993;2 Suppl 2(27):83-7.
73. Winawer SJ, Zauber AG, Ho MN, O'Brien MJ, Gottlieb LS, Sternberg SS, et al. Prevention of colorectal cancer by colonoscopic polypectomy. The National Polyp Study Workgroup. N Engl J Med 1993;329(27):1977-81.
74. Winawer SJ, Zauber AG, O'Brien MJ, Ho MN, Gottlieb L, Sternberg SS, et al. Randomized comparison of surveillance intervals after colonoscopic removal of newly diagnosed adenomatous polyps. The National Polyp Study Workgroup. N Engl J Med 1993;328(13):901-6.
75. Ahlquist DA, Shuber AP. Stool screening for colorectal cancer: evolution from occult blood to molecular markers. Clin Chim Acta 2002;315(1-2):157-68.
76. Osborn NK, Ahlquist DA. Stool screening for colorectal cancer: molecular approaches. Gastroenterology 2005;128(1):192-206.
77. Dong SM, Traverso G, Johnson C, Geng L, Favis R, Boynton K, et al. Detecting colorectal cancer in stool with the use of multiple genetic targets. J Natl Cancer Inst 2001;93(11):858-65.
78. Ahlquist DA, Skoletsky JE, Boynton KA, Harrington JJ, Mahoney DW, Pierceall WE, et al. Colorectal cancer screening by detection of altered human DNA in stool: feasibility of a multitarget assay panel. Gastroenterology 2000;119(5):1219-27.
79. Imperiale TF, Ransohoff DF, Itzkowitz SH, Turnbull BA, Ross ME. Fecal DNA versus fecal occult blood for colorectal-cancer screening in an average-risk population. N Engl J Med 2004;351(26):2704-14.